Luminex
complexity simplified.

Guava® Express 7-AAD

For Viability Determination

For Research Use Only
Not for Use in Diagnostic Procedures

PRODUCT DESCRIPTION AND INTENDED USE

The Guava® Express 7-AAD reagent can be used as a stand alone viability detector or in combination with antibodies or cellular stains.

Cells from a variety of sources may be assayed on the Guava Express, including in vitro cultured cells and peripheral blood leukocytes. A standard assay for Guava Express uses 1 x 10^5 cells in a 50 µL staining reaction. Assay results may be affected by the nonspecific uptake or binding of staining reagents by dead cells. To identify these events, the cell impermeant nucleic acid dye, 7-aminoactinomycin D (7-AAD) can be included in a Guava Express assay as an indicator of cell viability. 7-AAD is excluded from live, healthy cells but permeates dead and dying cells. Guava Express 7-AAD is provided as a convenient, ready-to-use solution, for use with the Guava Express phycoerythrin (PE)-conjugated secondary reagents.

MATERIALS PROVIDED

- Guava® Express 7-AAD (Catalog No. 4000-0061, 1.0 mL)

HANDLING AND STORAGE

1. Store the Guava® Express reagents refrigerated (2 to 8°C). Do not freeze. Refer to the expiration date on the package label. Do not use the reagent after the expiration date.
2. Guava Express 7-AAD is light-sensitive. Shield from excessive exposure to light.

WARNINGS AND PRECAUTIONS

1. The Guava® Express reagents are intended for research use only.
2. Wear proper laboratory attire (lab coat, gloves, safety glasses) when handling or using this reagent.
3. Exercise standard precautions when obtaining, handling, and disposing of potentially carcinogenic and mutagenic reagents.
4. The Guava Express reagents contain sodium azide, which is toxic. Contact with acids liberates toxic gas. Flush plumbing with copious amounts of water when disposing of azide compounds to avoid potentially explosive conditions arising from azide deposits in pipes.
5. Avoid microbial contamination, which may cause erroneous results.
6. All biological specimens and materials should be handled as if capable of transmitting infection and disposed of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Avoid specimen contact with skin and mucous membranes.
7. Exercise care to avoid cross contamination of samples during all steps of this procedure, as this may lead to erroneous results.
8. A Safety Data Sheet (SDS) is available from our website (*www.luminexcorp.com*) or by contacting Technical Support.

EQUIPMENT AND MATERIALS REQUIRED

- Guava® Instrument with GuavaSoft Software
- Guava Express 7-AAD
- Cell suspension for primary antibody labeling
- Phosphate buffered saline (PBS), or equivalent balanced salt solution, pH 7.2 to 7.4, supplemented with 5 mM EDTA and 0.05% sodium azide. (1 to 3% bovine serum albumin (BSA) can be added if desired). Buffer should not contain phenol red indicator and should be kept cold during staining.
- Micropipettors
- Disposable micropipettor tips
- Microcentrifuge tubes with screw caps, 1.5 mL (VWR, Catalog No. 20170-215 or equivalent), or
- Vortex mixer
- Centrifuge
- Disposable gloves
- 20% bleach solution
- Deionized water

BEFORE YOU BEGIN

1. Turn on the Guava® System.
2. Start GuavaSoft Software by double-clicking the GuavaSoft application icon on the desktop.
3. When initialization is complete, select Guava Express from the main menu. Allow the system to warm up for 15 minutes before running samples.

REAGENT AND SAMPLE PREPARATION

The following procedure is for dead cells. This staining can be combined as needed with other cellular staining protocols. Refer to the appropriate reagent package insert or contact Luminex for additional information. If you have a preferred procedure for antibody staining suitable for your application and cell types, prepare your samples accordingly.

Staining with Direct Conjugates and Express 7-AAD Reagent in Tubes

NOTE: Follow approved protocols for preparing suspensions from tissue samples or in vitro cultures, including methods for tissue fractionation, disruption, or release of adherent cells from culture substrates.

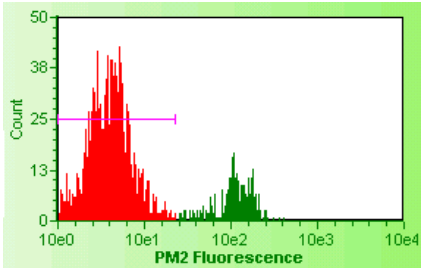
1. Pipette 1 x 10^5 cells, in 50 µL of buffer, into each microcentrifuge or sample acquisition tube per test. Include additional tubes for unstained, positive, and negative controls.
2. Add 5 µL of Express 7-AAD reagent to test samples and to appropriate control tubes.
3. Incubate for 30 minutes on ice or at 2 to 8°C.
4. Add 1 mL of cold PBS buffer to each tube and mix by gently vortexing. Centrifuge at 300 x g for 5 minutes. Carefully remove the supernatant without disturbing the cell pellet.
5. (Optional) Gently vortex each tube to disrupt the pellet, then add 500 µL of cold PBS buffer to each tube. Mix well.

Samples are ready for data acquisition on the Guava System. For details on acquiring sampler and analyzing the data, refer to the appropriate Guava System user's guide.

EXPECTED RESULTS

An example of staining results obtained using Guava Express 7-AAD is shown in Figure 1. Jurkat cells were washed with PBS and stained with Guava Express 7-AAD. The 7-AAD fluorescence histogram of acquired events with live cells (7-AAD negative) is shown in red and dead cells (7-AAD positive) is shown in green as differentiated by the marker.

Figure 1. 7-AAD fluorescence histogram of acquired events.



TROUBLESHOOTING TIPS

1. Mix each cell sample thoroughly on a vortex mixer before acquiring samples for consistent and accurate results.
2. If the concentration of the stained cell sample for data acquisition is high (≥ 5 x 10^5 cells/mL), the Guava® System may not yield accurate results. Dilute the sample further with additional PBS buffer (to 1 mL volume) to bring the cell concentration into an acceptable range.
3. Acquire data on stained cells soon after staining. Dilution of the reagents slows, but does not stop, continued staining of the cells. Prolonged exposure to 7-AAD may result in a higher background fluorescence. If acquisition is to be delayed beyond 60 minutes, wash the stained cell suspensions using cold PBS buffer. Keep washed samples chilled and protected from light until data acquisition begins.
4. If the background fluorescence seems high after staining, pellet the cells by centrifugation at 300 x g, carefully remove the supernatant and resuspend the pellet in 0.5 to 1 mL of PBS buffer. Reacquire the data from the sample. An additional wash with PBS buffer may reduce the background fluorescence further.
5. Run Guava Check using the Guava Check kit (Catalog No. 4500-0020) or easyCheck™ (Catalog No. 4600-3265) to verify proper instrument function and accuracy.
6. Always run Guava Clean or Quick Clean with 20% bleach and deionized water tubes after using the ViaCount™ Reagent and before running Guava

Express. Residual ViaCount Reagent may carry over into samples, affecting the results.

7. Periodically run Quick Clean using a deionized water tube (after every 20 to 25 sample acquisitions) to prevent a buildup from cell debris in the flow system. If your samples contain significant amounts of cellular debris, run Quick Clean more often to prevent clogs or blockage.
8. A clog or blockage of the flow system can be caused by cell aggregates, cell debris, bleach crystals, or other particulates. If you are acquiring data from a sample but the Cell Count number is not increasing and the Events to Acquire bar is not moving, there is probably a blockage of the flow system. Load a 20% bleach tube and click Backflush to flush out the clog. Load a deionized water tube and run Quick Clean to remove bleach residue. If this procedure does not alleviate the problem, consult the appropriate Guava System user's guide or contact Luminex for additional help.

For more troubleshooting tips, refer to the appropriate Guava System user's guide.

LIMITATIONS

1. The Guava® System will yield optimal results when the stained cell sample for acquisition is between 1 x 10^4 to 5 x 10^5 cells/mL. To obtain the most accurate assay results, adjust the concentration of the cell samples to within the recommended range.
2. Guava Express reagents are formulated to meet most assay requirements. Modification of the staining protocol and reagent concentration(s) may be necessary to ensure optimal performance for individual assays.

TRADEMARKS

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All other trademarks, including Microsoft and Excel, are trademarks of their respective companies.

For ordering information or technical support contact Luminex Corporation.

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